Atty. Dkt. No. 065691-0332
Appl. No. 10/632,101
**CONFIDENTIAL**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicants: Marco Ciufolini et al.

Title: 2-(3-aminoaryl)amino-4-aryl-thiazoles for the Treatment of Diseases

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Art Unit: 1626

**DECLARATION UNDER 37 CFR 1.132**

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

1. I, Marco A. Ciufolini, declare as follows:

2. I am one of the inventors of the captioned application.

3. My academic background and work experience are summarized in my *curriculum vitae*, which is attached as Exhibit A. Briefly, I started my independent career at Rice University, Houston, TX, in 1984, as an assistant professor of chemistry and rose to the rank of Full Professor in 1997. Being an internationally known authority in my scientific field, I was offered – and accepted – a chair of synthetic organic chemistry at the University of Lyon, France. I worked in Lyon from January 1998 until June 2004, when the University of British Columbia offered me a prestigious position as the Canada Research Chair in synthetic organic chemistry. Over the years, I have produced more than 90 research papers in synthetic organic chemistry and submitted 7 patents in the area of medicinal chemistry. I have directed the research of more than 30 Ph.D. students and 50 Master's students and founded or co-founded 3 companies (1 in the US, 2 in Europe) in which I serve, or I have served, as head of chemistry and in various other

capacities. I am a consultant for several industrial laboratories in the US and Europe. My research has been recognized with various scientific awards and it has earned me invitations to lecture at more than 180 technical meetings, industrial and academic research centers in North America, Europe and Asia.

4. The experiments described herein were conducted under my direct supervision and control.

5. The experiments described herein compare the activity of the claimed compounds to the compound "***5-methythio-4-(2-{[4-(phenylamino)phenyl]amino}(1,3-thiazol-4-yl))thiphene-2-carboxamidine***" disclosed by Illig et al (U.S. Patent 6,291,514) at col. 18 lines 25-26.

6. The activity of a compound is measured by its ability to inhibit cell proliferation and is expressed as a percentage inhibition of cell proliferation obtained in absence of treatment (% inhibition of proliferation). For example, the % inhibition of proliferation is zero (0) in absence of any treatment.

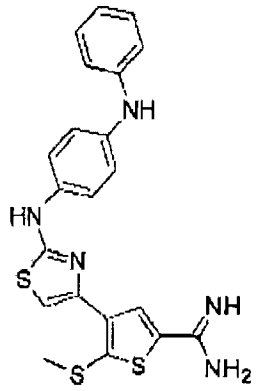
7. The $IC_{50}$ defines the concentration of compound necessary to obtain 50% inhibition of proliferation of a target cell. It should be noted that when a compound has little or no inhibitory activity, its $IC_{50}$ cannot be measured within a meaningful range of compound concentrations. For example, if a compound shows zero inhibition at the concentration of 1μM, its concentration to achieve 50% inhibition (if it could be achieved) will be too high to be meaningful for the testing, its $IC_{50}$ therefore is not available.

8. Specifically, the experiments described herein investigated the inhibitory effects, or lack thereof, that the tested compounds exhibited on different tyrosine kinases, including wild-type (WT) c-kit and mutant forms of c-kit. These experiments and their significance are summarized below.

## Activity of Cited Compound on Tyrosine Kinases

9. Illig generally relates to compounds for inhibiting urokinase, which is a proteolytic enzyme highly specific for a single peptide bond in plasminogen. Urokinase is a multidomain serine protease, which has a very different structure and properties compared to the stem-cell factor receptors, such as c-kit. The Illig compound is a 1,4-diamino derivative and also an amidine derivative known to be a protease inhibitor.

10. In one experiment, we tested the effects of the Illig compound shown below on a variety of tyrosine kinases, including several forms of c-kit.




***5-methythio-4-(2-{[4-(phenylamino)phenyl]amino}(1,3-thiazol-4-yl))thiphene-2-carboxamidine*** (Illig at col. 18 , lines 25-36)

11. Table 1 below shows Tyrosine kinase inhibitory activities of the Illig compound. The activities are expressed in % inhibition of cell proliferation at 1μM. Specifically, the following tyrosine kinases were tested:

(a) Ba/F3 is a murine hematopoietic cell line cultured in presence of IL-3. IL-3 is necessary for the survival and growth of Ba/F3 cells. IL-3 allows to rescue the cells when there are in contact with a potent and non toxic inhibitor. On the contrary, when cells die the inhibitor is considered toxic and unsuitable for therapy : (Ba/F3 +IL3)

(b) Ba/F3 expressing human c-kit receptor (wild type) cultured in presence of IL-3. IL-3 is necessary for the survival and growth of Ba/F3 cells. IL-3

allows to rescue the cells when there are in contact with a potent and non toxic c-kit inhibitor. On the contrary, when cells die the inhibitor is considered toxic and unsuitable for therapy: (Ba/F3 hKIT WT+ IL3)

(c) Ba/F3 expressing human c-kit receptor tyrosine kinase (wild type) cultured in presence of its ligand: SCF. SCF activates c-kit which in turns promotes cell proliferation. Inhibition of proliferation in presence of SCF means that the inhibitor is specific to c-kit since the same inhibitor does not inhibit the same cell line in the presence of IL3 (see paragraph b above) (Ba/F3 hKIT WT +SCF).

(d) Ba/F3 expressing murine c-kit (mutated in the enzymatic region [TK] at codon 814 and 816 respectively). Such mutation has been found in Acute Myeloid Leukaemia, Germ Cell Tumors and Mastocytosis. TK mutation constitutively activates c-kit which in turns promotes cell proliferation. Inhibition of proliferation in presence of SCF means that the inhibitor is specific to mutated TK c-kit. (Ba/F3 hKIT D814V and Ba/F3 hKIT D816V)

(e) Ba/F3 expressing murine c-kit (mutated in the juxtamembrane region [JM]). JM region in c-kit has been shown to be a negative regulatory region for c-kit activation. Such mutation has been found in GIST (gastro Intestinal Stromal Tumors). JM mutation constitutively activates c-kit which in turns promotes cell proliferation. Inhibition of proliferation means that the inhibitor is specific to mutated JM c-kit (Ba/F3 mKIT delta27)

(f) Ba/F3 expressing human Bcr-Abl. Bcr-Abl protein results from a reciprocal translocation between the breakpoint cluster region (bcr) and the tyrosine kinase domain of Abl kinase. This fusion has been identified in cases of Chronic Myeloid Leukaemia. Bcr proteins promote constitutive dimerisation of the Bcr-Abl chimeric proteins and lead to constitutive activity of Abl kinase which in turns promotes cell

proliferation. Inhibition of proliferation means that the inhibitor is specific to Abl kinase (Ba/F3 BCR-ABL)

(g) Ba/F3 expressing human FLT3 receptor tyrosine kinase (wild type) cultured in presence of its ligand : FL. FL activates FLT3 which in turns promotes cell proliferation. Inhibition of proliferation in presence of FL means that the inhibitor is specific to FLT3. (Ba/F3 hFLT3 WT +FL)

(h) Ba/F3 expressing human FLT3 receptor tyrosine kinase (mutated by a internal tandem duplication of the sequence in the juxtamembrane region [JM ITD]). JM region in FLT3 has been shown to be a negative regulatory region for FLT3 activation. Such mutation has been found in Acute Myeloid Leukaemia. They constitutively activates FLT3 kinase which in turns promotes cell proliferation. Inhibition of proliferation means that the inhibitor is specific to mutated JM FLT3. (Ba/F3 hFLT3 ITD)

(i) Ba/F3 expressing EGFR-FGFR1. EGFR-FGFR1 is a genetically engineered chimeric protein between the extracellular region of the EGFR and the tyrosine kinase domain of FGFR1 tyrosine kinase receptor. Addition of EGF ligand leads to dimerisation of the ligand binding domain of EGFR that lead to constitutive activity of FGFR1 kinase which in turns promotes cell proliferation. Inhibition of proliferation means that the inhibitor is specific to FGFR1 kinase. (Ba/F3 EGFR-FGFR1 +EGF)

(j) Ba/F3 expressing EGFR-FGFR3. EGFR-FGFR3 is a genetically engineered chimeric protein between the extracellular region of the EGFR and the tyrosine kinase domain of FGFR3 tyrosine kinase receptor. Addition of EGF ligand leads to dimerisation of the ligand binding domain of EGFR that lead to constitutive activity of FGFR3 kinase which in turns promotes cell proliferation. Inhibition of proliferation means that the inhibitor is specific to FGFR3 kinase. (Ba/F3 EGFR-FGFR3 +EGF)

(k) Ba/F3 expressing EGFR-PDGFR. EGFR-PDGFR is a genetically engineered chimeric protein between the extracellular region of the EGFR

and the tyrosine kinase domain of PDGFRβ tyrosine kinase receptor. Addition of EGF ligand leads to dimerisation of the ligand binding domain of EGFR that lead to constitutive activity of PDGFRβ kinase which in turns promotes cell proliferation. Inhibition of proliferation means that the inhibitor is specific to PDGFRβ kinase. (Ba/F3 EGFR-PDGFR +EGF)

(l) Ba/F3 expressing EGFR. EGFR is a tyrosine kinase receptor. Addition of EGF ligand leads to dimerisation of the ligand binding domain of EGFR that lead to activation of the kinase which in turns promotes cell proliferation. Inhibition of proliferation means that the inhibitor is specific to EGFR kinase. (Ba/F3 EGFR +EGF)

(m) Ba/F3 expressing Tel-Jak2. Tel-Jak2 is a genetic alteration that is the result of the fusion between the Jak2 tyrosine kinase gene and the TEL (ETV6) gene. This fusion has been identified in cases of human childhood T-cell acute lymphoblastic leukemia (ALL), pre-B cell and atypical chronic myeloid leukemia (CML). Tel-Jak2 fusion mutation results in constitutive Jak2 tyrosine kinase activity and has been shown to have oncogenic properties and promotes cell proliferation. Inhibition of proliferation means that the inhibitor is specific to Jak2 kinase. (Ba/F3 TEL-JAK2)

(n) Ba/F3 expressing Tel-Jak1. Jak1 is a cytoplasmic kinase from the same family as Jak2. Tel-Jak1 is a genetically engineered gene that is the result of the fusion between the Jak1 tyrosine kinase gene and the TEL (ETV6) gene. Tel-Jak1 fusion mutation results in constitutive Jak1 tyrosine kinase activity and has been shown to have oncogenic properties and promotes cell proliferation. Inhibition of proliferation means that the inhibitor is specific to Jak1 kinase. (Ba/F3 TEL-JAK1)

(o) Ba/F3 expressing Tel-Jak3. Jak3 is a cytoplasmic tyrosine kinase from the same family as Jak2.1dem as described for Jak1 above. (Ba/F3 TEL-JAK3)

(p) Ba/F3 expressing Tel-Tyk2. Tyk2 is a cytoplasmic tyrosine kinase from the same family as Jak2. Idem as described for Jak1 above. (Ba/F3 TEL-TYK)

(q) Ba/F3 expressing H4-Ret. H4-Ret is a genetic alteration that is the result of the fusion between the Ret tyrosine kinase region of the receptor gene and the 55 KDa nuclear and cytosolic protein encoded by H4 gene. This fusion has been identified in cases of human thyroid papillary carcinomas. H4-Ret fusion results in constitutive Ret tyrosine kinase activity, It has been shown to have oncogenic properties and promotes cell proliferation. Inhibition of proliferation means that the inhibitor is specific to Ret kinase. (Ba/F3 H4 RET)

**Table 1. TYROSINE KINASE INHIBITORY ACTIVITIES OF THE ILLIG COMPOUND**

| | *Illig Compound* |
|---|---|
| *Ba/F3 hKit +IL3* | 0 |
| *Ba/F3 m/hKit +SCF* | 0 |
| *Ba/F3 mKit D814/ hKitD816* | 0 |
| *Ba/F3 mKit Delta27* | 0 |
| *Ba/F3 TEL-JAK1* | 0 |
| *Ba/F3 TEL-JAK2* | 0 |
| *Ba/F3 TEL-JAK3* | 0 |
| *Ba/F3 TEL-TYK* | 0 |
| *Ba/F3 H4-RET* | 0 |
| *Ba/F3 BCR-ABL* | 0 |
| *Ba/F3 hFLT3WT* | 0 |
| *Ba/F3 hFLT3ITD* | 0 |
| *Ba/F3 EGFR-FGFR1* | 0 |
| *Ba/F3 EGFR-FGFR3* | 0 |

| | |
|---|---|
| *Ba/F3 EGFR-PDGFR* | 0 |
| *Ba/F3 EGFR* | 0 |

12. The results show that the Illig compound has no inhibitory activity on any type c-kit tested. In addition, the results demonstrate that the Illig compound is not selective toward any types of c-Kit.

**Activity of Claimed Compounds on Tyrosine Kinases**

13. To compare the effects of the claimed compounds to the Illig compound, we conducted experiments testing the potency and selectivity of the claimed compounds. Specifically, we tested numerous compounds which represent structural diversities across the entire scope of the amended claims.

14. The results of these tests are show in Table 2:

**Table 2. TABLE OF BIOLOGICAL ACTIVITIES OF THE CLAIMED COMPOUNDS**

**Legend:** (-) $IC_{50}$ greater than 1000 nM
(+) $IC_{50}$ greater than 100 nM but no more than 1000 nM
(++) $IC_{50}$ no more than 100 nM

| R1 | Compound Example Number | Ba/F3 hKit +IL3 | Ba/F3m hKit +SCF | Ba/F3 mKitD814/ hKitD816 | Ba/F3 mKit Delta27 | Ba/F3 BCR-ABL |
|---|---|---|---|---|---|---|
| CO-NRR' | 009 | - | + | - | ++ | - |
| | 010 | - | + | - | ++ | - |
| | 011 | - | + | - | + | - |
| | 012 | - | ++ | - | ++ | - |
| | 013 | - | + | - | ++ | - |
| | 014 | - | + | - | - | - |
| | 015 | - | ++ | - | | - |
| | 016 | - | + | + | + | - |
| | 017 | + | + | + | ++ | + |
| | 018 | - | - | + | - | - |
| | 019 | - | + | - | ++ | - |
| | 020 | - | ++ | - | ++ | + |

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| R1 | Compound Example Number | Ba/F3 hKit +IL3 | Ba/F3m hKit +SCF | Ba/F3 mKitD814/ hKitD816 | Ba/F3 mKit Delta27 | Ba/F3 BCR-ABL |
|---|---|---|---|---|---|---|
| | 021 | - | + | - | ++ | - |
| | 022 | - | - | - | + | - |
| | 023 | - | + | - | ++ | - |
| | 024 | + | ++ | + | ++ | + |
| | 025 | + | ++ | - | ++ | - |
| | 026 | + | + | + | ++ | + |
| CO-OR | 098 | - | - | - | + | - |
| | 097 | - | - | - | - | - |
| | | | | | | |
| CO-R | 004 | - | - | - | ++ | - |
| | 002 | - | + | - | ++ | - |
| | 005 | - | - | - | ++ | - |
| | 003 | - | + | - | + | - |
| | 028 | - | ++ | + | ++ | - |
| | 103 | - | + | - | ++ | - |
| | 067 | - | + | - | ++ | ++ |
| | 074 | - | + | - | ++ | + |
| | 077 | - | + | - | ++ | - |
| | 076 | + | - | + | + | + |
| | 078 | - | + | - | ++ | ++ |
| | 079 | - | + | - | ++ | + |
| | 105 | - | + | - | ++ | - |
| | 108 | - | - | - | ++ | - |
| | 117 | - | - | - | ++ | - |
| | 110 | - | - | - | ++ | - |
| | 080 | - | + | - | ++ | + |
| | 027 | - | - | - | - | - |
| | 081 | - | ++ | - | ++ | - |
| | 116 | - | - | - | + | - |
| | 084 | - | + | - | ++ | - |
| | 122 | - | - | - | ++ | - |
| | 124 | - | - | - | - | - |
| | 006 | - | - | - | ++ | - |
| | 111 | - | - | - | ++ | - |
| | 118 | - | - | - | ++ | - |
| | 113 | - | - | - | ++ | - |
| | 068 | - | - | - | + | - |
| | 069 | - | + | - | ++ | - |
| | 070 | - | - | - | + | - |
| | 071 | - | - | - | + | - |
| | 029 | + | ++ | + | ++ | + |
| | 030 | - | + | - | ++ | - |
| | 031 | - | + | - | + | - |
| | 032 | - | + | - | | - |

| R1 | Compound Example Number | Ba/F3 hKit +IL3 | Ba/F3m hKit +SCF | Ba/F3 mKitD814/ hKitD816 | Ba/F3 mKit Delta27 | Ba/F3 BCR-ABL |
|---|---|---|---|---|---|---|
| | 033 | + | ++ | - | ++ | + |
| | 034 | + | + | + | | + |
| | 035 | + | + | + | | + |
| | 036 | - | + | - | ++ | - |
| | 037 | - | + | - | ++ | - |
| | 072 | - | + | - | ++ | - |
| | 038 | - | - | - | + | - |
| | 039 | - | - | - | + | + |
| | 040 | + | - | + | + | - |
| | 041 | + | + | + | + | + |
| | 042 | + | ++ | + | ++ | + |
| | 043 | + | ++ | + | ++ | + |
| | 099 | - | + | - | + | - |
| | 061 | - | ++ | - | ++ | + |
| | 065 | + | ++ | + | ++ | + |
| | 100 | + | ++ | + | ++ | + |
| | 086 | + | + | + | + | + |
| | 101 | + | + | + | ++ | + |
| | 102 | + | - | + | + | + |
| | 058 | + | ++ | + | ++ | + |
| | 056 | + | ++ | + | ++ | + |
| | 054 | - | ++ | - | ++ | + |
| | 044 | + | - | - | + | + |
| | 088 | + | ++ | + | ++ | + |
| | 045 | - | ++ | - | ++ | - |
| | 062 | + | + | + | ++ | + |
| | 104 | + | ++ | + | ++ | + |
| | 047 | + | ++ | + | ++ | + |
| | 107 | - | + | - | ++ | + |
| | 048 | + | ++ | - | ++ | - |
| | 055 | - | ++ | + | ++ | + |
| | 049 | + | ++ | - | ++ | + |
| | 050 | + | ++ | - | ++ | + |
| | 087 | - | - | - | ++ | + |
| | 075 | - | - | - | ++ | ++ |
| | 089 | - | + | - | ++ | - |
| | 051 | + | + | - | ++ | + |
| | 082 | - | + | - | ++ | + |
| | 090 | - | + | - | ++ | - |
| | 083 | - | + | - | ++ | + |
| | 060 | - | - | - | + | - |
| | 059 | - | + | - | ++ | - |
| | 085 | - | - | - | ++ | + |
| | 052 | - | + | - | ++ | - |

| R1 | Compound Example Number | Ba/F3 hKit +IL3 | Ba/F3m hKit +SCF | Ba/F3 mKitD814/ hKitD816 | Ba/F3 mKit Delta27 | Ba/F3 BCR-ABL |
|---|---|---|---|---|---|---|
| | 046 | + | - | - | ++ | - |
| | 073 | - | - | - | + | - |

15. Most of the tested compounds showed low $IC_{50}$ in the Ba/F3m hKit+SCF assay and the Ba/F3 mKit Delta27 assay, while they showed high $IC_{50}$ in the rest of the assays. The tested compounds which are representative of the claimed compounds demonstrated a high potency and specificity to wild-type c-kit and/or mutants, unlike the Illig compound.

16. This high degree of potency and specificity has important practical applications. For example, the specificity allows the claimed compounds to be used to treat diseases characterized in expression of c-kit, whether wild-type or a mutant form. For example, the claimed compounds could be used in the treatment of mast cell-dependent diseases[1] in which mast cells abnormally proliferate upon activation of c-kit, including inflammatory or auto-immune diseases, as well as in certain type of tumour associated with the expression of activated JM c-Kit mutants, such as Gastrointestinal Stromal Tumour ("GIST").

## Conclusion on the Biological Testing

17. The claimed compounds are unique in their properties and differ from the Illig compound in ways that were not expected. For example, the claimed compounds demonstrated a high specificity to wild-type c-Kit and/or mutants. On the other hand, the Illig compound fails to demonstrate any inhibitory activity on c-kit and specificity. These results were unexpected and surprising. Illig provides no motivation to alter its compounds to arrive at the claimed compounds. Indeed, Illig et al does not relate to inhibition of c-Kit.

[1] See specification at page 1, line 24 to page 2, line 9.

**Meaning of "pendant basic nitrogen functionality"**

18. A chemist of ordinary skill in this field would ready understand what "pendant basic nitrogen functionality" refers to. The term "pendant basic nitrogen functionality" is of common usage in the field. "Pendant" means the moiety is attached to the rest of the molecule by a single bond, e.g., the attached moiety is not attached as a fused ring. The term "basic nitrogen functionality" refers to a moiety containing a nitrogen atom, wherein the nitrogen atom can be protonated. Thus, the nitrogen atom imparts to the moiety the property of being "basic." A person of ordinary skill in the field could thus readily determine whether a given moiety is a "pendant basic nitrogen functionality."

19. That a person of ordinary skill in the field would readily understand the term "pendant basic nitrogen functionality"—and that the term reflects common usage—is demonstrated by the following excerpts (with emphasis in bold and underlined). The excerpts reflect the common usage of the term "pendant . . . functionality" referring to a functional group within a moiety, and the common usage of "basic nitrogen functionality" to refer to a moiety having a basic nitrogen.

20. In U.S. Pat. 6753434, "As a further example, a poly(butadiene) having **pendant carboxylic acid functionality** can react with the hydroxyl functionality on either of the hydroxyl ..." (col. 3, lines 53-54).

21. In U.S. Pat. 4470859, "For perfluorocarbon copolymers having **pendant sulfonyl fluoride functionality**, crystallized PTFE-like material begins to appear in the copolymer at between ..." (col. 6, lines 28-29).

22. In U.S. Pat 4064161, the title is "Polymers having **pendant acrylate and methacrylate functionality**" (Title).

23. In US Pat. 6686350, "The first compound comprises a IIB/IIIa specificity determinant, comprising a **basic nitrogen functionality**, which, for example, ..." (col. 4, line 54).

24. In US Pat. 5126370, "It should be appreciated that when the R1 residue in D contains a **basic nitrogen functionality**, it must be used in a protected form and then deprotected ..." (col. 8, lines 16-17).

25. In U.S. Pat. 4237038, "... alpha, beta-ethylenically unsaturated the monomer containing a **basic nitrogen functionality** ..." (Col. 1, line 21).

26. "Pendant" means hanging or suspended (see http://www.m-w.com/dictionary/pendant). In the context of a molecule, "pendant" means the moiety is attached by a bond rather than, e.g., as a fused ring, to the remainder of the molecule.

27. I hereby declare that all the statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful false statements so made are punishable by fine or imprisonment, or both, under Section 101 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

August 9, 2007
Date

Marco A. Ciufolini